綜 説

Insecticide-resistance and Darwinism. * A. W. A. BROWN (Professor and Head of the Department of Zoology, University of Western Ontario, London, Canada. Temporarily, Scientist (Biologist) of W. H. O., Division of Environmental Sanitation). Received May 8, 1957. *Botyu-Kagaku* 22, 277, 1957.

48. 殺虫剤抵抗性とダウイニズム (抄訳: 防虫科学 22, 282, 1957)

Next summer, the International Congress of Zoology will meet in London to celebrate the centenary of "The Origin of Species," and to honour its author Charles Robert Darwin. Darwin stated, 100 years ago, that it was natural selection, operating on the normal variation of animals and plants which over the course of time separated the subspecies which, he said, were the origin of species. With that book he made his outstanding contribution to the progress of science and radically changed the thought of man. For proof, Darwin pointed not only to what could be seen in different parts of the world, but also to what happened in history. In England, during at least the previous 200 years, man had been developing the various breeds of domestic animals and cultivated plants which man wanted, by selecting those individuals which were desirable and rejecting those that were substandard. This is selection for man's good.

During the last twenty years we have succeeded quite well in doing the reverse, selection for man's embarrassment, selection to man's detriment. We have brought about the selection of new strains which are now resistant to the chemicals which were used to control them, whether antibiotics to kill bacteria or insecticides to kill the insects which compete with man for his food or transmit his diseases.

The best example of insecticide-resistance, of course, is that of the housefly to DDT. The first discovery was made in 1946 at Arnas in northern Sweden, only 2 years after the introduction of DDT for housefly control. DDT-resistance then appeared in 1947 south of Rome, Italy, and in 1948 in the state of New York, U. S. A. Since then, the same phenomenon was discovered in houseflies in every part of the world, including Hikone in 1954.

The housefly has also developed resistance to other insecticides, which like DDT are in the class known as chlorinated hydrocarbons. In 1949, BHC-and dieldrin-resistance was reported in California. Finally resistance has appeared to those insecticides which we at first thought never induce it, namely the organophosphorus compounds; it was reported from Denmark in 1955 that parathion, diazinon and Resitox can no longer control houseflies there.

This resistance is not only confined to the housefly. DDT-resistance was shown by *Culex molestus* in 1947 in Italy, and in 1948 by two species of *Aedes* salt-marsh mosquitos in Florida; it also appeared in the bed-bug *Cimex* in 1948, and *Pulex* fleas in 1949. In the winter of 1950-51, in Korea, it became impossible to control body lice *Pediculus* with DDT any more. DDT-resistance in lice is particularly serious in eastern Asia because of the danger of typhus. In the same year it became evident that the lack of control of malaria mosquitos *Anopheles* in Greece was not due to inadequacies in spraying, nor to the chemicals being substandard, but to a change on the part of the insects themselves; they had now become resistant to DDT.

Subsequently DDT-resistance of *Anopheles* mosquitoes appeared in two other regions, Java and Saudi Arabia. At this point the World Health Organization became vitally concerned, because one of its main activities has been to promote the control of the insects which transmit the great endemic diseases, of which malaria is the greatest.

In addition, malaria mosquitoes began to show resistance to dieldrin in 1955 with *Anopheles quadrimaculatus* in Mississippi in U. S. A. and *Anopheles gambiae* in Nigeria in Africa. Meanwhile cockroaches have developed chlordane-resistance, which first appeared in Texas in 1952

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and is now very common in the U. S. A. Cattle ticks, *Boophilus decoloratus* and *B. microplus*, had developed BHC-resistance in South Africa and Australia around 1948.

Not only insects of medical and veterinary importance, but also agricultural insects have developed resistance. In the last few years, DDT-resistance has appeared in four species of caterpillars: the codling moth *Carpocapsa pomonella* in the U.S.A. and South Africa, the diamond-back moth *Plutella maculipennis* in Java, the cabbage worm *Pieris rapae* in several states of the U.S.A. and probably in Japan, and the cabbage looper *Trichoplusia ni* in New York State. DDT-resistance also developed in three species of leaf-hopper *Erythroneura*, at least one species of plant bug *Lygus* on alfalfa, and the potato flea beetle *Epitrix cucumeris*. Some agricultural pests have developed resistance to parathion. No less than five species of tetranychid mites have developed resistance to parathion in various parts of the U. S. A. with isolated records from Germany and South Africa. And parathion-resistance has been detected in five species of aphids, including the common *Myzus persicae*.

The resistance of insects to insecticides was first observed in 1908 and reported in 1914 by Melander with the San José scale, *Aspidiotus perniciosus*, in the state of Washington, U.S.A., with the failure of lime-sulphur sprays to exert the customary control. He showed that this resistance was a peculiarity of the strain which had developed in the Clarkson valley. Shortly after, in California between 1912 and 1925, three species of scale on citrus developed resistance to hydrogen cyanide fumigation. These are the black scale *Saissetia oleae*, California red scale *Aonidiella aurantii* and citricola scale *Coccus pseudomagnoliarum*. In 1928 the codling moth, *Carpocapsa pomonella*, one of the most expensive pests in agriculture, developed resistance to lead arsenate in Colorado. Arsenic-resistance in both species of cattle ticks and in the peach twig borer *Anarsia*, and tartar-emetic-resistance of the citrus thrips *Scirtothrips*, completed the earlier history.

The recent agricultural calamities include chlordane-resistance in the wireworm *Conoderus falli*, endrin-resistance in the spiny bollworm *Earias*, rotenone-resistance in the Mexican bean beetle *Epilachna varivestris*, and toxaphene-resistance in the cotton leafworm *Alabama argillacea* and the bollworm *Anthonomus grandis*.

For some people insect-resistance means simply figures in account books. To others it means a change of insecticides. But to biologists, of cource, it means that we must look at the insects to see what makes them resistant, what is it about them that is different from the normal susceptible strains.

When DDT-resistance was first discovered in the housefly at Arnas, the difference was considered to lie in their thicker and darker tarsi. In fact, when the resistant strain was found near Rome in Italy, it was thought to be a subspecies of *Musca domestica* and was described as *M. domestica tiberina*. But soon afterwards, similar DDT-resistance bagan to be reported from different countries and different places. So the characteristic could not be ascribed to certain natural subspecies. Many laboratories found that if took a normal strain, they could develop, by selection with DDT, their own resistant strain from the normal susceptible strain. It therefore becomes clear how these resistant strains have appeared. They have been produced by a process of natural selection, or rather "unnatural selection". If you expose a population of flies to DDT, the insecticide kills the most susceptible ones, and leaves some to mate and lay eggs. If you rear the offspring of the survivors, it is found that this second generation is more resistant on the average than the previous one, and if you repeat the process generation after generation the level of resistance will steadily increase until you have a strain which is wholly resistant.

Usually the average level of resistance increases slightly at first and more steeply later, rising in an exponential curve to reach a maximum at perhaps the twentieth generation or so. Twenty generations of the fly in most parts of the world means about 2 years, and it has been found indeed that DDT-resistance usually follows DDT spraying in approximately 2 years.

It must be stressed that the resistant strain can only be produced if you select; in other words, you must kill some of the flies. All attempts to produce a resistant strain by exposing successive generations to truly sub-lethal dosages have failed. It is clear that resistance cannot be explained by the doctrine of J. B. Lamarck, that a given individual can adapt itself and, having adapted itself, hand on to its offspring that same adaptation. In fact, it has been found that insects do not adapt themselves to DDT in the first place; pretreatment with small doses does not make them any more tolerant of larger doses later. Therefore we are forced to the conclusion, paradoxical at first sight, that the adaptation which causes resistance was not produced by the insecticide. There is no post-adaptation as a consequence of the chemical, but rather a pre-adaptation which the chemical exploited. You can produce the resistant strain by killing the most susceptible ones and selecting the less susceptible ones (in which the pre-adaptation already exists) to produce the next generation. This is in full accord with Darwin's theory of evolutionary change through natural selection and survival of the fittest.

When you have succeeded in getting your resistant strain of housefly, can you discern any difference between a resistant fly on the one hand and a susceptible fly on the other? If you make studies comparing flies of one resistant strain with flies of one susceptible strain, you will probably detect some differences between them. But if you check the result by taking several resistant strains and several susceptible ones, you will find that the differerences which you first thought true are no longer valid in general.

Firstly, there is no morphological difference characteristically distinguishing flies of resistant strains from susceptible ones. Wiesmann discovered morphological peculiarities in the Swedish resistant flies found at Arnas in 1946, in that they were darker, and had thicker cuticle of tarsi and pulvilli than houseflies at Basle in Switzerland. The DDT-resistant flies which appeared later in other parts of the world never showed any abnormal morphology. It is therefore now considered that the characteristics he found are probably those of flies from northern Sweden and not of the resistant strain. It is now concluded that there is no difference in appearance, in anatomy or morphology between a resistant strain and a susceptible one.

Although there is a tendency for individuals which grow more slowly to be more resistant than those which grow faster within a group, if you take a resistant strain as a whole and compare it with a susceptible one, there is no significant difference in their bionomics. They have almost the same life-cycle.

There is no difference in the behaviour between resistant strains and susceptible ones. There are some who say that the resistant flies characteristically refuse to rise to walls treated with DDT, but the fact probably is that the resistant flies are seen resting on the untreated ground because they are intoxicated but surviving. In short, this behaviour is a response to the insecticide, and not a case of difference in the intrinsic behaviour.

Furthermore, there is no consistent difference in oxygen consumption, and no consistent difference in cytochromoxidase content, between resistant strains and susceptible ones. Nor is there any characteristic difference in cholinesterase activity. There is a tendency for resistant strains to contain more fat, of a lower melting point, than susceptible ones, and it may be that resistant strains contain more Cu than susceptible ones.

It might be thought that flies of resistant strains absorb less DDT than susceptible ones. But on the whole resistant strains absorb DDT on the same rate as susceptible strains. In fact, if you compare the resistance by injecting DDT into the body of the fly instead of applying it by contact, you will find the same difference between them in their response to DDT.

Well, there is one difference which so far has proved to be constant. The resistant strain can detoxify DDT. Houseflies of DDT-resistant strains strip HCl from the molecule of DDT, leaving behind DDE; in other words, they dehydrochlorinate DDT to DDE. It is true that some susceptible strains can do this too, but only slightly. Of course, susceptible strains can only withstand slight amount of DDT, and if they get a little more they die and can no longer dehydrochlorinate. But the difference between resistants and susceptibles is a real one, in that at parallel dosages the resistants can dehydrochlorinate at a much faster rate.

Dehydrochlorination is an activity of the living organism, and you can designate it as an enzyme process. Indeed, it has been possible to separate and purify enzyme preparations about 120 times, the dehydrochlorination proceeding *in vitro*. This enzyme was discovered by Sternburg and Kearns of the University of Illinois, and has been purified and characterized as DDT-dehydrochlorinase by Moorefield. One of the most remarkable points is that, in the housefly, it requires to be activated by glutathione. When so activated, it has optimum temperature of 37°C and an optimum pH of 7.4. It can only utilize certain compounds as substrates, namely the *p,p'*-substituted diphenylethane derivatives such as DDT, DDD, methoxychlor and DFDT. Moorefield has found that this enzyme after purification consists of 4 separate proteins.

Resistance to BHC, on the other hand, follows a different mechanism. True, gamma-BHC is first dehydrochlorinated to pentachlorocyclohexene, but the enzyme DDT-dehydrochlorinase is not involved. In this case, the fly tissue rather quickly metabolizes it further to water-soluble compounds.

Thus there are two different mechanisms of detoxification. Indeed, it will be found that if you induce DDT-resistance, you have no induced BHC-resistance. The DDT-resistant *Anopheles* are perfectly susceptible to BHC and dieldrin. On the other hand, BHC-resistant cattle ticks are perfectly susceptible to DDT. Dieldrin-resistant *Anopheles gambiae* and *A. quadrimaculatus* are also perfectly susceptible to DDT.

If you cross DDT-resistant flies with susceptible ones, the F_1 hybrid offspring will be on the whole intermediate in resistance. The F_2 generation shows a wide range of variation all the way from susceptibility to resistance, some being as susceptible as the susceptible grandparent and some being as resistant as the resistant. Since this range is fairly gradual and does not show any segregation into different categories of resistance, it was at first reasonably concluded that DDT resistance was due to a number of genes acting together; in short, that resistance was polygenic. However, with more recent improvement in genetical technique, the use of single-pair mating, and greater precision in the categorization of the F_2, segregation has been found to occur in the F_2, and it is now concluded that one main gene determines DDT-resistance in the housefly. Since this gene is not on the X-chromosome, it is on one of the other 5 autosomes. Whether the same gene is involved everywhere remains to be seen.

Several workers have found that their resistant strains contain many individuals with abnormalities in the wing venation. Some veins may be reticulated, other interrupted. Certain investigators have found that resistant strains have broader 2nd abdominal sternites than susceptible strains. But at present, we have no reason to believe that these characters are linked with DDT-resistance. Certainly we have no reason to consider that they are an expression of a pleomorphic gene for DDT-resistance.

The gene for DDT-resistance in the Hikone strain of *Drosophila melanogaster* has been shown by Tsukamoto and Ogaki to derive chiefly from a locus on chromosome II between 66 and 67. They have also found the gene for nicotine-resistance to reside on chromosome III near the spindle-fibre attachment. These two genes show an interaction and may play a part in general resistance. These resistant genes have been shown by Oshima and Hiroyoshi to occur in *D. virilis* also. It is noteworthy that DDT-resistance in *Drosophila* does not involve dehydrochlorination.

In other instances, there may be small genes which aid up to a resistance which is usually not specific in the insecticides it concerns. In other words, these genes altogether produce what may be called "vigor tolerance". The best example of vigor tolerance is the lead-arsenate resistance of the codling moth which appeared in Colorado in 1928. These larvae were resistant to lead-arsenate simply because they did not die so easily from desiccation, not from lead arsenate, and thus could wander around the apple looking for an arsenic-free spot for a longer time before dying. It is a characteristic of vigor tolerance that the resistance involves insecticides in general, not just special insecticides. Conversely, it bears no specific relation to the chemical that induces it, and may be induced by selection without chemicals at all.

It has been shown recently that houseflies can develop resistance to organo-phosphorus compounds, but it is not nearly as strong as the DDT-resistance we know, the greatest increase being only about 20 times the normal. It is quite possible that the resistance to organophosphorus compounds may be vigor tolerance. Indeed, organophosphorus-resistant flies have become much more resistant to chlorinated hydrocarbons, to which they never been exposed. We have one selective agent, the insecticide S. 17, which does not induce resistance to itself but induces resistance to organic phosphates and still more to chlorinated hydrocarbons, whereas to S. 17 itself the flies remain completely susceptible. It also appears that parathion is very good at inducing resistance to other organophosphorus compounds rather than to itself.

If the resistance is due to a detoxifying enzyme, and if those individuals which produce that enzyme have a particular gene to produce the enzyme, where does this gene come from? Did DDT itself produce the gene? The answer is "No". DDT does not, like nitrogen mustard, induce mutations. Fruit-flies which have grown in sublethal amounts of DDT for 50 generations did not increase their mutation rate. We are led to the conclusion that presumably the gene must have been there from the first; in other words, somewhere among the fly population some flies contained the gene. But they are very scarce indeed, and you don't know of their existence in the first place. What you are up against, again, is not post-adaptation but pre-adaptation.

In the last year, the correctness of this hypothesis has been demonstrated. The insect is *Anopheles gambiae* and the chemical involved in the resistance is dieldrin. In 1954 a house-spraying programme to control this malaria mosquito was started in Northern Nigeria on the southern edge of the Sahara desert, where nobody had ever seen any synthetic chemical, let alone dieldrin. In November 1955, these mosquitoes were observed surviving on sprayed walls, and a sample was taken for test by a standard method. The test showed that the population had become, on the average, 8 times more resistant than the normal one from unsprayed regions. Then, a sample of eggs of this strain was collected in the village of Ambursa in the sprayed area, and they were air-mailed to London so that Davidson could establish a laboratory colony. On arrival, only about 5% of the eggs hatched, but the larvae grew well and emerged into very healthy adults.

When Davidson had obtained a sufficient number of mosquitoes to test the resistance of this colony to dieldrin, he found that instead of 8 times they were now 800 times as resistant as normal *A. gambiae* from Lagos in Northern Nigeria. Then Davidson took his resistant Ambursa strain and crossed it with the susceptible Lagos strain. He found that the hybrids showed intermediate resistance. Since F_1 hybrid males sterile. Davidson backcrossed the F_1 females with the resistant parent, and found that 50% of the offspring were as resistant as the resistant parent and 50% showed the intermediate resistance characteristic of the F_1 hybrid. He made the other backcross with the susceptible parent and found that 50% of the offspring were as susceptible as the susceptible parent and 50% showed the intermediate resistance. These results constituted proof that the dieldrin-resistance was monofactorial, that is due to allelism in a single gene.

It is now possible to classify any individual *A. gambiae* as homozygous resistant (RR), heterozygous hybrid (Rr) or homozygous susceptible (rr), by using diagnostic mortality-test dosages. In September 1956 a party went into Northern Nigeria to test the genotype composition in nature, and found in the dieledrin-sprayed zone now that about 90% of individuals in the population were homozygous resistant types. But the most interesting point of all is that in one of the unsprayed areas 0.04% of the mosquitoes were of the hybrid type, and in another as many as 6% of the individuals were heterozygous for the resistant gene.

Thus we see that individuals carrying the genes for resistance already existed in Northern Nigeria before dieldrin arrived on the scene in 1954. By killing only the susceptible genotypes the dieldrin selection pressure increased the proportion of resistant individuals, until dieldrin-resistance characterized the entire population. A similar situation existed in the bed-bug *Cimex hemipterus* on Formosa, where DDT-resistance developed within 2 years in a population which before spraying contained 1 resistant individual in every 200; and evidently the same kind of thing occurred with our houseflies.

And so with the proven pre-existence of resistant genes in certain individuals of the population we have the source of that variation in susceptibility upon which selection can act to produce a strain with the new characteristic of resistance. In insecticide-resistance therefore, 100 years after the publication of "The Origin of Species", we have a perfect example of the truth of Darwin's main hypothesis.

動植物の自然に見られる変異に働く自然淘汰が種の起源であるというのがDarwinの説でありますが，これをわれわれ人間が役に立つ方向に使つて家畜や農作物の品種を作り出しています．しかし近年になつて逆にわれわれに有害なバクテリヤや昆虫において，薬品に抵抗性のある品種が出来つゝあります．殺虫剤に対するイエバエの抵抗性品種は DDT に対して 1946年にはじめて発見され，その後いろいろの塩素化合物に認められるに至りました．その後，イエカ，ヤブカ，ハマダラカ，シラミ，ノミ，ナンキンムシの DDT に対する抵抗性，羊ダニの BHC 抵抗性，ゴキブリの chlordane 抵抗性などが認められるに至りました．最近には蛾，ヨコバイ，アブラムシ，甲虫などの DDT 抵抗性，ハダニの parathion 抵抗性なども現われました．

抵抗性品種は通常のものと形態的にも生活史や行動の上にも，普通の酵素の量においても，またDDTの表皮透過性においても変りません．たゞDDTを解毒してDDEとするDDT-dehydrochlorinaseを抵抗性品種がもつている点が違つています．BHC, dieldrin, chlordane に対する抵抗性は DDT 抵抗性とは違つた生理的機構によつているようです．

DDT で淘汰を続けると DDT 抵抗性のものが生じてくるのですが，もしもまつたく死亡率が0であるならば，決して抵抗性品種は出来て来ません．後適応はおこりませんし，Lamarck 説のような遺伝は認められません．抵抗性の遺伝は正統の遺伝学に従つていて，イエバエに見られるDDT抵抗性は大体単因子遺伝であります．ショウジョウバエでは抵抗性遺伝子が第2染色体上にあることまでわかつています．DDT 自身は突然変異をひきおこしませんが，抵抗性因子をもつた個体を選び出します．このことは最近北ナイジェリヤで行われたハマダラカの一種 *Anopheles gambiae* の dieldrin 抵抗性についての野外と実験室内の研究によつてよく示されています．殺虫剤抵抗性こそはダウィニズムの正当さを示す完全な例証であります．(内田俊郎抄)

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